

# Spatio-temporal birth/death dynamics of membrane nanoclusters in cell signaling

M. Ángeles Serrano,<sup>1</sup> Manuel Jurado,<sup>2</sup> and Ramon Reigada<sup>2</sup>

<sup>1</sup>*Departament de Física Fonamental, Universitat de Barcelona,  
Martí i Franquès 1, 08028, Barcelona, Spain*

<sup>2</sup>*Departament de Química Física, Universitat de Barcelona,  
Martí i Franquès 1, 08028, Barcelona, Spain*

(Dated: December 20, 2012)

## Abstract

We present a minimal motif model for transmembrane cell signaling. The model assumes signaling events taking place in spatially distributed nanoclusters regulated by a birth/death dynamics. The combination of these spatio-temporal aspects can be modulated to provide a switch-like response behavior without invoking sophisticated modeling of the signaling process as a sequence of cascade reactions and fine-tuned parameters. Our results also show that the spatial organization of the signaling process and the fact that the distributed events take place in nanoclusters with a finite lifetime regulated by local production is sufficient to obtain a robust and high-fidelity response.

Transmembrane cellular signaling pathways are responsible for linking external stimuli and internal cellular actions. Typically, signaling molecules activate specific receptor proteins on the cell membrane triggering a cascade of interactions that diffuse messengers inside the cell regulating its activity, growth and development. Signaling pathways not only sense information but integrate and process it into fast (seconds to minutes) and robust high-fidelity responses [1]. The importance of certain ubiquitous motifs in signaling pathways—such as activation cascades and feedback loops—for this processing is well recognized. How spatio-temporal mechanisms influence signal transduction is much less understood. In particular, discretization of spatial structures can explain striking behaviors of biological sensing circuits, like for instance macroscopic synchronization of clustered oscillating bacteria colonies impossible in bulk [2].

The interplay between cascades and feedback loops can explain intriguing properties such as opposing cell fate decisions depending on different stimuli provoking different activation amplitudes [3–7]. As a striking example, the mitogen-activated protein kinase (MAPK) pathway governs crucial cellular processes like proliferation and differentiation [8], and its dysfunction has been related with cancer [9]. Mathematical models have shown that, in the absence of feedback regulation, the MAPK cascade elicits a steep response to the input signal if successive protein activations are performed in a distributive manner [10], while feedback regulations modulate the overall sensitivity of the pathway [11, 12].

Recently, membrane nanoclusters concentrating signaling proteins have been proposed as a new fundamental mechanism to modulate and increase the efficiency and specificity of the MAPK cascade. The work by Harding and Hancock [13] has revealed that activated receptor proteins aggregate in the membrane forming nanoclusters, that recruit the cascade downstream factors and perform signal transduction. These signaling platforms display two important spatio-temporal characteristics: they are transient with short lifetimes (typically under 1s), and dispersed in the cell membrane occupying a small reaction volume (radii  $\approx 10nm$ ) [13]. When the nanocluster spatial organization is combined with cascades and feedback loops, *in silico* and *in vivo* analyses of MAPK signaling show that the system is able to perform high-fidelity signal transduction in a range of parameters where nanoclusters work independently as switches, responding maximally to very low input signals [14]. The intricacy of the cascade and high number of tuned-for-ultrasensitivity parameters in those *in silico* approaches hinder to discriminate the role of the spatio-temporal nanocluster dynamics

in signaling output.

In this Letter, we consider a remarkably simple and generic motif model that incorporates the aggregation of signaling proteins into discrete transient domains, but that flattens the pathway structure to unveil the importance of the spatio-temporal dynamics of membrane nanoclusters. Interestingly, we find that complex behaviors attributed to the particular architecture –cascades of distributed activations and feedback loops– of signaling pathways can be also achieved by regulating the spatio-temporal dynamics of nanoclusters encapsulating much simpler signaling motifs. More specifically, ultrasensitivity is found to be affected by the lifetime of nanoclusters. To explore how cells might have protected the fidelity of signal transduction, two scenarios have been compared: a situation where the nanocluster lifetime is externally regulated, and the case where it is not prefixed but linked to their local activity. In the latter case, we report that self-regulation induces robust individual switch-like behavior and high fidelity global responses for a wide range of kinetic parameters.

The simplest way to model the complex features of nanoclusters signal transduction is to model each nanocluster as a minimal signaling motif based on the standard Michaelis-Menten formulation for enzymes,



where  $E$ ,  $S$ ,  $ES$  and  $P$  stand for the enzyme, substrate, intermediate and product species, respectively, and  $k_1$ ,  $k_{-1}$  and  $k_2$  are reaction rate constants. The role of  $E$  is assigned here to the nanocluster platform where the activity of the signaling motif takes place. Typically, nanocluster platforms are transient structures assembled by anchored proteins in the inner leaflet of plasma membrane. These can be proteins like GTPase Ras, that become activated by mediation of the cytoplasmatic protein SOS as a catalyst when the external stimulus (f.i., growth factor) binds to membrane receptors. In the context of MAPK signaling (Fig. 1a) the first reversible reaction in Eq. (1) corresponds to the recruiting of Raf protein to immobile Ras nanoclusters and its subsequent activation, which starts the MAPK cascade, whereas the second (catalytic) step comprehends successive phosphorylation and activation of MEK and ERK kinases.

In our model, signaling nanoclusters are assumed to follow a dynamics that controls their number and lifetime. We first consider a birth/death mechanism that does not depend on the spatial distribution or the functioning of nanoclusters, but represents some extrinsic

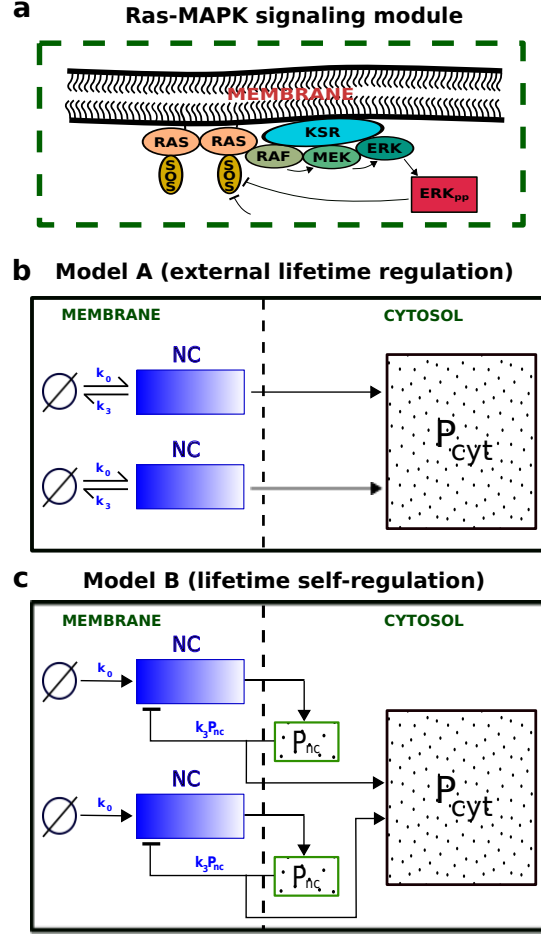


FIG. 1: (Color online) a) Sketch of the Ras-MAPK signaling platform. Two different nanocluster disassembling mechanisms are proposed. b) Schematic representation of model A for nanocluster signaling with external lifetime regulation. c) Schematic representation of model B for nanocluster signaling with a negative backward loop controlling nanocluster lifetime. In panels b) and c) only two nanoclusters are represented, but the number in our simulations is of hundreds.

regulation (model A, Fig. 1b). Nanoclusters are dynamically formed in the cell membrane at rate  $k_0$ , whereas, independently, a fixed constant rate  $k_3$  determines the frequency of nanocluster disassembling. During their lifetime, signaling nanoclusters can generate product molecules according to the reaction motif in Eq. (1). The external stimulus is represented by parameter  $\alpha$ , that is set to unity at maximal stimulus. The role of  $\alpha$  is two-fold. First, in vivo experiments reveal that nanocluster generation is proportional to input growth factor concentration [14], so we consider the frequency of nanocluster formation to be proportional to the stimulus,  $k_0 = \alpha k_0^m$ , where  $k_0^m$  corresponds to nanocluster generation rate at maximal

stimulus. Second, kinase phosphorylation is known to happen in two separated encounters, both promoted by stimulus, instead of occurring sequentially in a single encounter [10]. Such a distributive mechanism is accounted in our signaling motif by setting  $k_2 = \alpha k_2^m$ , with  $k_2^m$  being the catalytic rate at maximal stimulus.

In the numerical simulations, all events corresponding to nanocluster birth and death, and to internal molecular transformations, are treated as stochastic events because of the small numbers of proteins involved and the limited lifetime of signaling platforms. In particular, we consider Poissonian processes with a frequency determined by the corresponding reaction rates, that can be found in literature. For the MAPK pathway, cytosolic concentration of Raf is about  $10^{-7}M$  [10], whereas Raf activation is of order  $\approx 10^6 - 10^8 M^{-1}s^{-1}$  [6, 15], which leads to a forward reaction rate  $k_1 \approx 0.1 - 10s^{-1}$ . The dissociation reaction is slower,  $k_{-1} \approx 10^{-2}s^{-1}$  [6, 15]. Catalytic constant rates corresponding to kinase phosphorylation processes are much larger,  $k_2 \approx 10 - 100s^{-1}$  [6, 14, 15]. Finally, death rate  $k_3 = 2s^{-1}$  is tuned to adjust the estimated  $0.5s$  average lifetime of Ras nanoclusters [14]. Activation frequency at maximal stimulus,  $k_0^m$ , is arbitrarily fixed to  $1000s^{-1}$ , so that the average number of simulated nanoclusters is of the order of a few hundreds. This number is well below the typical maximum number of Ras nanocluster in a cell ( $\approx 50000$ ) [14] but assures a sufficient statistical ensemble for our stochastic simulations in a reasonable computational time.

According to the proposed model A, individual signaling nanoclusters can generate both graded and switch-like outputs (see. Fig. 2), depending on the relative values of the reaction rates and whenever nanoclusters do not die too fast,  $k_1 \lesssim k_3$ . If the limiting reaction is the formation of product molecules from the intermediate complex  $ES$ ,  $k_1 > k_2$ , nanoclusters generate a graded signal since the impact of the distributive mechanism in the formation of product  $P$  through  $k_2 = \alpha k_2^m$  becomes relevant. In contrast, for experimentally determined reaction rates  $k_1 < k_2$ , and the limiting reaction is the formation of  $ES$ . In this situation, once the intermediate complex is formed, product molecules are generated very fast quite independently of the contribution of the stimulus in the catalytic step. Then, a nanocluster responds maximally to very low inputs (ultrasensitivity) and its output is switch-like, presenting a steeper response for greater differences between  $k_1$  and  $k_2$ . These behaviors are summarized in Fig. 2a, that reports results from simulations run with a fixed number of operating nanoclusters; namely, for a nanocluster generation rate  $k_0$  independent of the input

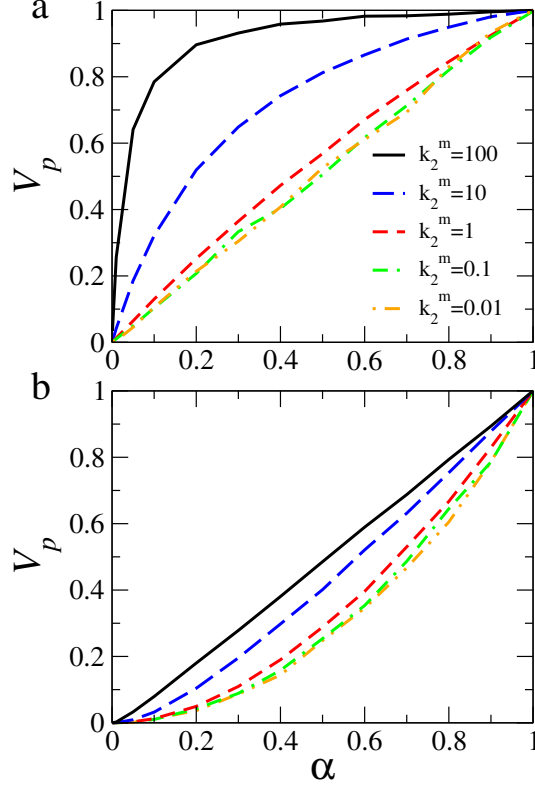


FIG. 2: (Color online) Global response  $V_P$  as a function of stimulus  $\alpha$  in model A (external lifetime regulation), for  $k_1 = 1s^{-1}$ . a) Number of nanoclusters fixed ( $k_0 = k_0^m$ ). b) Number of nanoclusters regulated by stimulus ( $k_0 = \alpha k_0^m$ ).

stimulus and fixed to  $k_0^m$  in order to average a large ensemble of signaling platforms. The velocity  $V_P$  of  $P$  formation normalized with respect to the maximal production is represented as a function of input stimulus  $\alpha$  for different values of  $k_2^m$ , given  $k_1 = 1s^{-1}$ .

The global response of the system corresponds to the integration of local signaling events taking place in different activated nanoclusters. In cell membrane, the number of nanoclusters is proportional to stimulus concentration,  $k_0 = \alpha k_0^m$  [14]. Figure 2b shows that this global response is nonlinear when the nanoclusters function in the graded regime (low values of  $k_2^m$ ), while the whole-cell system generates a graded nearly linear product output in the regime of nanoclusters working as nanoswitches (large values of  $k_2^m$ ), meaning that in this case the signaling response is directly proportional to input stimulus. The fact that nanoclusters respond maximally even at low stimulus yields to a global response only dependent on the number of nanoclusters, proportional to stimulus. This implies that the cell system of ultrasensitive nanoswitches achieves high-fidelity signal transduction by performing an

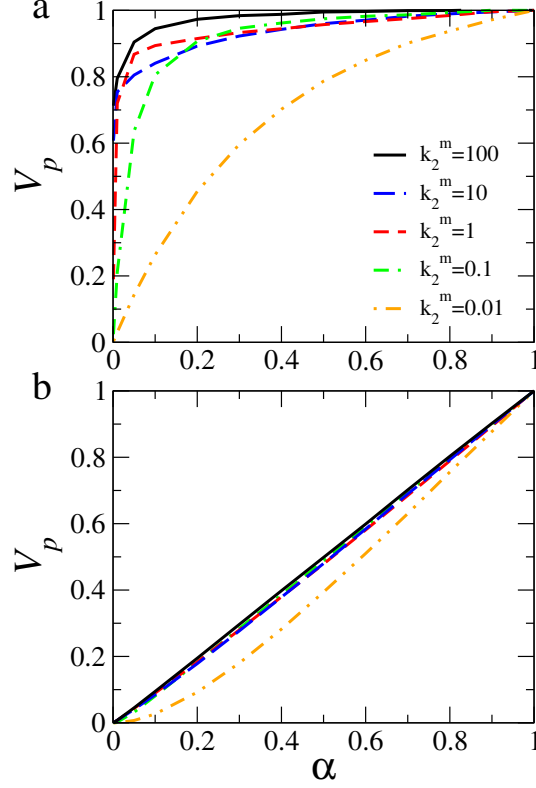


FIG. 3: (Color online) Global response  $V_P$  as a function of stimulus  $\alpha$  in model B (lifetime self-regulation), for  $k_1 = 1s^{-1}$ . a) Number of nanoclusters fixed ( $k_0 = k_0^m$ ). b) Number of nanocluster regulated by stimulus ( $k_0 = \alpha k_0^m$ ).

analogue-digital-analogue transmission [14], an obvious advantage that could be one of the reasons for the active compartmentation of cell signaling processes. We note that our results are obtained for the simplest internal structure of the signaling pathway given in Eq. (1), without the recursion to intricate reactions profuse in biological details.

As an alternative to model A, we propose a signaling mechanism where the nanocluster lifetime is self-regulated via the coupling of nanocluster disassembly to its local activity, without the intervention of external factors (model B, Fig. 1c). We assume that product molecules remain transiently accumulated in a local pool that acts as an internal clock exerting a negative impact in the lifetime of the nanocluster. The more it has produced, the higher its chances to die, as it happens for instance in Ras-MAPK signaling: production of ERK promotes the phosphorylation of the SOS factor, which inhibits the activity of Ras aggregates [7, 8] (Fig. 1a). The concept of local pool is acceptable for short nanocluster lifetimes of fractions of second. Then, the restricted diffusive motion of product species does

not allow them to travel far from the signaling membrane complex. We model the death rate of a nanocluster as  $k_3 P_{nc,i}(t)$ , where  $k_3$  is a constant rate and  $P_{nc,i}(t)$  is the number of signaling product molecules in the local pool generated by the nanocluster  $i$  up to time  $t$ . The latter expression is the simplest dependence for a negative upstream inhibition due to the final product protein and corresponds to the opposite limit to model A. Whereas model A considers that the dynamics of signaling platforms is completely regulated externally, in model B this regulation is absolutely modulated by their local activity.

To investigate the impact of local activity regulating the death of nanoclusters, we study the response of model B for the same parameter values used in the simulations of model A (Figs. 2a and 2b). Lifetime self-regulation enhances nanocluster sensitivity, maintaining the switch-like response for a wider range of values of  $k_2^m$ , see Fig. 3a for a fixed number of signaling platforms ( $k_0 = k_0^m$ ). The explanation is related to the fact that in model B nanoclusters can be active for longer periods when the input signal is low. In model A, most nanoclusters are disassembled before producing any  $P$  molecule when working under a low stimulus. In these cases, and in particular when  $k_2^m$  is small, the effect of the input stimulus in the production of  $P$  becomes critical. Instead, in model B, the death probability of a nanocluster is zero until it has produced at least one  $P$  molecule, so that the second step in Eq. (1) is no longer critical even at low values of  $k_2^m$ . Notice that the lifetimes of nanoclusters are longer in model B at low input stimulus, they approach the lifetime fixed in model A ( $k_3^{-1}$ ) at moderate stimulus, and may become even shorter at maximal stimulus. As a consequence of the gain in nanocluster sensitivity, the global response fidelity is also enhanced in model B, as it is shown in Fig. 3b for simulations using  $k_0 = \alpha k_0^m$ .

We have also performed a systematic study of signal transmission fidelity in both models at different values of the kinetic rates  $k_1$  and  $k_2^m$ . Fidelity of signal transduction is quantified here by parameter  $\phi$ , computed as the integral of the global response rescaled by the number of nanoclusters,  $\frac{V_P}{\alpha}(\alpha)$ , for the whole range of input stimulus. High-fidelity transduction is achieved when the system response,  $V_P$ , correlates to stimulus,  $\alpha$ , so  $\phi \rightarrow 1$ . This requires nanoclusters to work in the range where they behave as ultrasensitive nanoswitches. Figure 4 presents the values of  $\phi$  in the  $(k_1, k_2^m)$  parameter space spanning two orders of magnitude for each kinetic rate. Notice that high-fidelity signal transmission is a robust feature for the signaling mechanism involving a self-regulated nanocluster dynamics (model B), whereas for the scheme based on a fixed nanocluster lifetime (model A) such virtue requires a fine



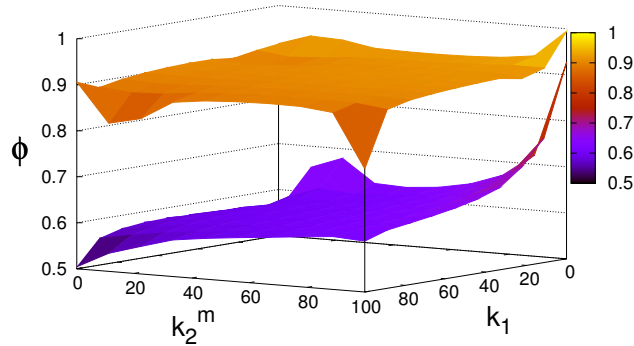


FIG. 4: (Color online) Signal transmission fidelity  $\phi$  computed at different values of  $k_1$  and  $k_2^m$  for models A (bottom surface) and B (top surface). Simulations using  $k_0 = \alpha k_0^m$ .

tuning of the reaction kinetic parameters.

Two important conclusions can be derived from our simulations. First, the sensitivity of signaling platforms is found to be modulated by their lifetime, so it is not exclusively determined by the particular architecture of the signaling pathway as suggested so far. Second, comparison of two extreme models for nanocluster disassembly reveals the importance of the physical origin of nanocluster lifetime regulation. As two extreme possibilities, we propose model A where nanoclusters lifetime is externally regulated by a fixed frequency, and model B where individual nanocluster activity fully determines its duration. Most likely, biological cell signaling may be regulated by a mixture of these two extreme situations. Importantly, we have shown that any contribution to nanocluster lifetime regulated by local production promotes robust individual ultrasensitivity outputs and, consequently, high-fidelity global responses for a wider range of reaction kinetic rates as compared to model A. Therefore, it could be conjectured that nanocluster lifetime self-regulation protects the signaling response from variability in the particular architecture of the signaling structure and in the rates of involved reactions.

Many modeling approaches have been attempted to describe cell signaling processes but the complexity of the signaling structure and the number of kinetic parameters hide the particular role of nanocluster dynamics. In this Letter, we propose a simple and generic signaling motif model that captures essential features of signal transduction, such as ultrasensitiveness and fidelity, and could apply to different types of spatial signaling domains

following a temporal birth/death dynamics. Nevertheless, the observed complexity of signaling pathways may entail some biological advantages, such as enabling plasticity to modulate different response amplitudes triggering opposing cell fate decisions within a single cell. In the context of our framework for nanocluster cell signaling, we plan to tackle these questions and others, such as the processing of time dependent stimulus, in future research.

This work was supported by Generalitat de Catalunya grant Ref. 2009SGR1055; the Ramón y Cajal program of MICINN; MICINN Project BFU2010-21847-C02-02.

- 
- [1] U. S. Bhalla and R. Iyengar, *Science* **283**, 381–387 (1999).
  - [2] A. Prindle, *et al.*, *Nature* **481**, 39–44 (2011).
  - [3] F. A. Brightman and D. A. Fell, *FEBS Letters* **482**, 169 (2000).
  - [4] W. de Ronde, F. Tostevin, and P. R. ten Wolde, *Phys. Rev. Lett.* **107**, 048101 (2011).
  - [5] P. Andreu-Pérez, *et al.*, *Science Signaling* **4**, 190 ra58 (2010).
  - [6] B. Schoeberl, C. Eichler-Jonsson, E. D. Gilles and G. Müller, *Nature Biotechnology* **20**, 370 (2002).
  - [7] S-Y. Shin, *et al.*, *J. Cell Sci.* **122**, 425 (2008).
  - [8] W. Kolch, *Biochem. J.* **351**, 289 (2000).
  - [9] A. S. Dhillon, S. Hagan, O. Rath and W. Kolch, *Oncogene* **26**, 3279 (2007).
  - [10] C-Y. F. Huang and J. E. Ferrell, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10078 (1996).
  - [11] B. N. Kholodenko, *Eur. J. Biochem.* **267**, 1583 (2000).
  - [12] O. E. Sturm, *et al.*, *Science Signaling* **3**, 153 ra90 (2010).
  - [13] A. S. Harding and J. F. Hancock, *Trends in Cell Biology* **18**, 364 (2008).
  - [14] T. Tian, A. Harding, K. Inder, S. Plowman, R. G. Parton and J. F. Hancock, *Nature Cell Biology* **9**, 905 (2007).
  - [15] W. Kolch, *et al.*, *Science Signaling* **3**, ra90 (2010).